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SYNTHESIS, CHARACTERIZATION, THERMAL, ANTICANCER AND DNA BINDING PROPERTIES OF Co(II), Ni(II), Cu(II), Cd(II) AND Zn(II) COMPLEXES WITH SCHIFF BASE

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ABSTRACT

A series of metal complexes of Co(II), Ni(II), Cu(II), Cd(II) and Zn(II) were synthesized with newly prepared ligand. This ligand was prepared by the condensation of hydrazide with 2-chloro benzaldehyde. The resulting complexes were characterized by elemental analyses, spectroscopic data i.e. IR, UV, mass, molar electric conductibility as well as magnetic measurements. The complexes were square planar and octahedral in nature. Thermal studies of the complexes were also reported. Metal complexes exhibited DNA binding, cleavage and anticancer activities which were significantly better than the ligand.

KEYWORDS: Schiff Base, Thermal, Angiogenesis, DNA Binding, Metal Complexes

INTRODUCTION

Metal complexes derived from Schiff bases occupy an important position in the development of coordination chemistry, as observed from their synthesis, structure, reactivity and physico-chemical properties **(Z. Shirin et al., 1992)**. Transition metal complexes of Schiff base ligands have been extensively investigated for many years due to their potential applications in many fields (**T. Glaser et al., 2006)**. Transition metal complexes of azomethines have witnessed a great deal of interest in the recent years because of their chemical, pharmacological **(V. M. Leovac et al., 1983; S. Padhye et al., 1985)** and analytical applications **(R. B. Singh et al., 1991)**.The incorporation of metals into the organic entity results in an electron rich environment due to the electronic states inherent in the metal centers and increased aspect ratio of metal core, which enhance the thermal stability of liquid crystal phase. This property can be exploited to develop promising molecular materials for technological applications like electrooptical memory devices **(Liu C Y et al., 1997)**, solar cell (**Gregg B A et al., 1989; Schmid M L et al., 2001**), one-dimentional energy transport **(Markovitsi D et al., 2003)** and semiconductors **(Ohta K et al., 2003)**. In addition, the presence of nitrogen and oxygen donor atoms in the complexes act as stereospecific catalyst for many reactions like oxidation **(R. I. Kureshy et al., 1999)**, reduction **(Y. Aoyama et al., 1986)** and hydrolysis **(T. Daniel Thangadurai et al., 2002)**.A number of Schiff base complexes **(K. Drabent et al., 2004; M.H. Klingele et al., 2003; V.B. Arion et al., 2003; M. Mashaly et al., 1999)** have been tested for antibacterial activities and they have been found antibacterial **(A.S. Kabeer et al., 2001; A.H. El-Masry et al., 2000; P.G. More et al., 2001; S.N. Pandeya et al., 1999)**, antifungal **(P.G. More et al., 2001; S.N. Pandeya et al., 1999; W.M. Singh., 1988)**,

herbicidal **(S. Samadhiya et al 2001)** and anti cancer **(S.B. Desai, P.B. Desai., 2001; P. Pathak., 2000)** activities. The necessity of angiogenesis for continued tumor growth has led to the development of alternative strategies for treating cancer based on the selective inhibition with the growth of tumor microvessels **(Folkman J et al., 1995).** Cancer, the second cause of death in the world, is continuing to be a major health problem in developing as well as in under developed countries **(S. Eckhardt et al., 2002).** Planning and development of anticancer drugs with fewer or no side effects are important for the treatment of cancer. The search for such potential anticancer drugs has led to the discovery of synthetic molecules with anti-carcinogenic activity.

Survey of literature demonstrates that interest on the design of novel transition metal complexes capable of binding and cleaving duplex DNA with high sequence and structure selectivity **(B.H. Geierstanger et al., 1994; C. Liu et al., 1996; G. Pratviel et al., 1995)** increase continuously. There has been an increasing focus on the binding study of small molecules to DNA during the last decades, since deoxyribonucleic acid (DNA) is an important genetic substance in organisms **(K.E. Erkkila et al., 1999; J.K. Barton et al., 1984; A. Chouai et al., 2005)**. Errors in gene expression can often cause diseases and play a secondary role in the outcome and severity of human diseases **(J. Hooda et al., 2006)**. Since DNA is particularly sensitive to oxidative cleavage, vast majority of the studies on metallonucleases have focused mainly on the molecules that cleave DNA oxidatively. The worldwide real success of cisplatin as an anticancer drug has stimulated interest in the synthesis of a wide range of transition metal complexes with potential anticancer activity **(S.E. Livingstone et al., 1980)**. Such complexes have been found to be useful for the design and development of compounds that can restrict certain enzymes. So a more complete understanding of DNA-drug binding is valuable in the rational design of DNA structural probe, DNA footprinting and sequence-specific cleaving agents **(F. Liang et al., 2004; J.M. Kelly et al., 1985)**. In order to develop new drugs which specifically target DNA, it is necessary to understand the different binding modes which a small molecule is capable of undergoing. The recognition modes for the noncovalent binding of small molecules to DNA are intercalative binding, groove binding and external electrostatic binding **(G.M. Zhang et al., 2004; S. Sharma et al., 2005).** Among these interactions, intercalation and groove binding are the most important DNA-binding modes as they invariably lead to cellular degradation. A number of metal chelates, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents, have been used as probes of DNA structure in solution **(S. Mahadevan et al., 1997; S.J. Lippar et al., 1978; S.M. Hech et al., 1986)**. Additionally, the metal ion type and different functional groups of ligands, which are responsible for the geometry of complexes, also affect the affinity of metal complexes to DNA. So the investigation on the interaction of the Schiff-base transition metal complexes with DNA has a great significance for disease defense, new medicine design and filtration and clinical application of drugs.

In continuation of research work by our group on coordination compounds **(BushraBeguma et al., 2013**) herein we undertake the synthesis of novel ligand and Co(II), Ni(II), Cu(II), Cd(II) and Zn(II)complexes from hydrazide and 2-chloro benzaldehyde and to study their DNA binding, anticancer activities. The results of this work are being reported in this paper.

EXPERIMENTAL SECTION

Materials and Methods

All the chemicals used in the preparation of Schiff base and its metal complexes were of AR grade. The solvents were distilled before use. For the preparation and analyses, distilled water was used.

A Perkin-Elmer CHN analyzer (model 2400) was used for C, H and N analyses. The room temperature molar conductance was determined using a Century digital conductivity meter (model cc 601) with a dip type cell and a smooth platinum electrode. The electronic absorption spectra of the complexes were recorded as dilute solutions on a Shimadzu 160A/240A UV-visible spectrophotometer. The ^{1}H NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz with TMS as the internal standard. Mass spectra were obtained with a VG70-70H spectrophotometer. The infrared spectra of the solid samples were recorded in the range 4000-500 cm^{-1} on a Perkin-Elmer 597/1650 spectrophotometer using KBr pellets. The magnetic moments were measured out using gouy balance. The TG-DTA experiments were carried out in air using a Shimadzu DT-40 thermal analyzer. The heating rate employed was 10˚C per minute and platinum cups were used to hold about 5 mg of the samples. Purity of the compoundwas checked by TLC.

SYNTHESIS OF SCHIFF BASE AND ITS METAL COMPLEXES

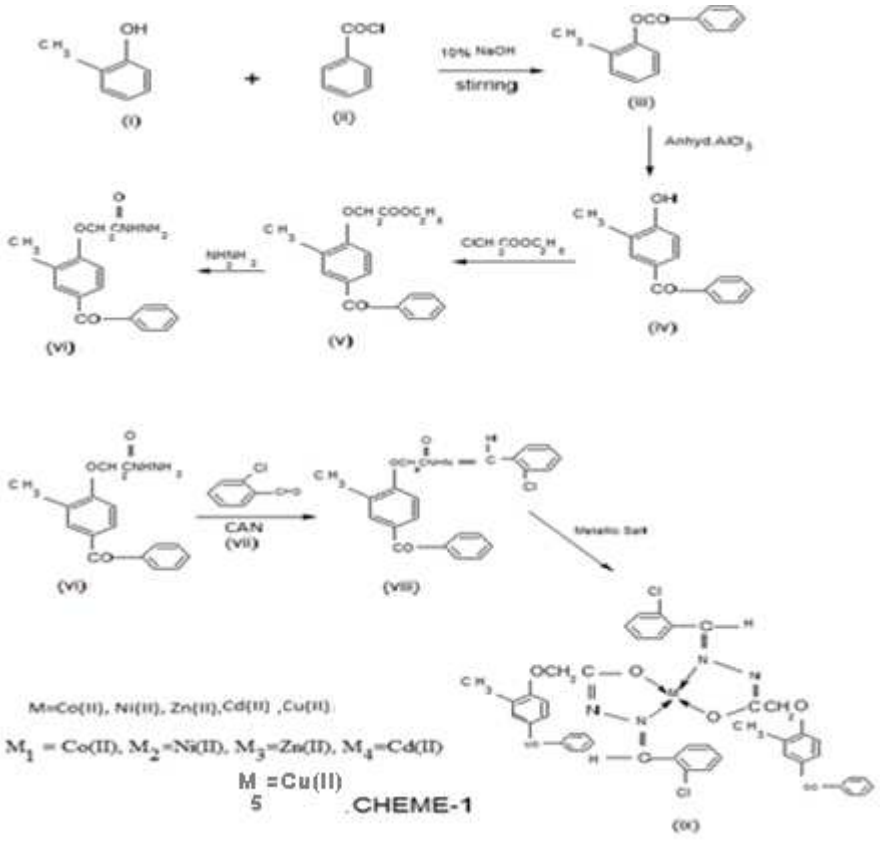

Figure 1

General Procedure for the Synthesis of Schiff Base: (viii)

A mixture of acetohydrazide (**vi,** 1 mmol), 2-chlorobenzaldehyde (**vii,** 1 mmol) and ceric ammonium nitrate (0.25 mmol) in ethanol was heated under reflux with stirring for one hour and poured in ice cold water. The white

precipitate was formed. Thiswas filtered off and washed with ethanol. The product was recrystallized from hot ethanol and stored over anhydrous $CaCl_2$. The purity of ligand was checked by thin layer chromatography and elemental analysis.

Viii: Yield 80%; M.p. 120-122^oC; IR (Nujol): 1645(C=O), 1618 cm^{-1} (C=N), 3266 cm^{-1}(N-H); ^1H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 5.3 (s, 2H, OCH_2), 6.9-8.6 (m, 8H, Ar-H), 11.8 (bs, 1H, CONH), 8.4 (s, IH, CH). $C_{23}H_{19}N_2O_3Cl$ (406):C, 67.91; H, 4.67; N, 6.88. Found: C, 68.01; H, 4.17; N, 6.90%.

General Procedure for the Synthesis of Complexes (ix)

A solution of Schiff base (**viii,** 1 mmole) and metallic salt (0.5 mmole) in methanol was refluxed for 2 hours. The product was filtered off and washed with hot ethanol. The product (**ix**) was recrystallized from a mixture of chloroform and ethanol (4:1). The product was stored over anhydrous $CaCl_2$.

(M_1): Yield 70-74%; M.p. >250; IR (Nujol): 1610 cm^{-1} (>C=N-N=C<), 651 cm^{-1} (M-N), 517 cm^{-1} (M-O). $C_{48}H_{36}N_4O_6Cl_2Co$ $(H_2O)_2$ (930): C, 61.94; H, 4.33; N, 6.01. Found: C, 61.90; H, 4.22; N, 6.11%, Molar conductance: 21.54, Magnetic moment: 3.82-3.90 B M.

(M_2): Yield 70-74%; M.p. >250; IR (Nujol): 1607 cm^{-1} (>C=N-N=C<), 579 cm^{-1} (M-N), 519 cm^{-1} (M-O). $C_{48}H_{36}N_4O_6Cl_2Ni$ (894): C, 64.46; H, 4.05; N, 6.26. Found: C, 64.70; H, 4.02; N, 6.32%, Molar conductance: 27.20, Magnetic moment: 2.82-2.96B M.

(M_3): Yield 70-74%; M.p. >250; IR (Nujol): 1608 cm^{-1} (>C=N-N=C<), 590 cm^{-1} (M-N), 566 cm^{-1} (M-O). $C_{48}H_{36}N_4O_6Cl_2Zn$ (901): C, 63.98 45; H, 4.02; N, 6.21. Found: C, 64.0; H, 4.11; N, 6.01%, Molar conductance: 11.10.

(M_4): Yield 70-74%; M.p. >250; IR (Nujol): 1604 cm^{-1} (>C=N-N=C<), 596 cm^{-1} (M-N), 546 cm^{-1} (M-O). $C_{48}H_{36}N_4O_6Cl_2Cd$ (948): C, 60.81; H, 3.82; N, 5.91. Found: C, 60.01; H, 4.01; N, 5.98%, Molar conductance: 13.20.

(M_5): Yield 70-74%; M.p. >250; IR (Nujol): 1605 cm^{-1} (>C=N-N=C<), 598 cm^{-1} (M-N), 529 cm^{-1} (M-O). $C_{48}H_{36}N_4O_6Cl_2Cu$ (947): C, 64.11; H, 4.03; N, 6.23. Found: C, 64.23; H, 4.11; N, 6.15%, Molar conductance: 23.20, Magnetic moment: 1.88-1.93 B M.

BIOLOGY

Shell Less Chorioallantoic Membrane Assay

Antiangiogenic effect of Schiff base and its metal complexes were studied according to the method of Auerbach et al **(Auerbach R., Kubai L., Knighton D., Folkman J, 1974)**.

Fertilized hens eggs were surface sterilized using 70% alcohol. The eggs were incubated in fan assisted humidified incubator at 37ºC. On the 4th day, the eggs were cracked out into thin films of the hammock within a laminar flow cabinet and were further incubated. On the day 5th when blood vessels were seen proliferating from the center of the eggs within the hammock, filter paper discs loaded with 100 µg ofLigand and its metal complexes were placed over the proliferating blood vessels and the eggs were returned to the incubator. Results for antiangiogenic effect of the compound were observed after 24 hours.

DNA CLEAVAGE EXPERIMENTS

For the gel electrophoresis experiment, the solution of complexes in DMF (1mg/mL) was prepared and test samples (1µg) were added to the CT-DNA samples and incubated for 2h at 37˚C. Agarose gel was prepared in TAE buffer (4.84g Tris base, PH 8.0, 0.5M EDTA/l. pH 7.3); the solidified gel attained at approximately 55˚C was placed in electrophoresis chamber flooded with TAE buffer. After that 20µL of each of the incubated complex-DNA mixtures (mixed with bromophenol blue dye at 1:1 ratio) was loaded on the gel along with standard DNA marker and electrophoresis was carried out under TAE buffer system at 50 V for 2h. At the end of electrophoresis, the gel was carefully stained with EtBr (Ethedium bromide) solution (10µg/mL) for 10-15 min and visualized under UV light using a Bio-Rad Trans illuminator. The illuminated gel was photographed by using Polaroid camera (a red filter and Polaroid film were used).

RESULTS AND DISCUSSIONS

Chemistry

The ligand(viii) was prepared by condensation reaction of acetohydrazide(vi) with 2-chlorobenzaldehyde. TLC spots suggested the formation of the ligand. The complexes (M_1-M_5) were formed by the direct reaction of the ligand with cobalt(II), nickel(II), copper(II), zinc(II) and cadmium(II) chloride.

The elemental analysis (**Table 1**) of the compounds is in good agreement with their proposed formulae and the conductance values indicate the non-electrolytic nature of the complexes (**Geary W J et al., 1971**). The magnetic measurements of the complexes at room temperature reveal that all the complexes are paramagnetic and diamagnetic, consistent with octahedral and square planar geometry of cobalt (II), nickel (II), zinc (II), cadmium (II) and copper (II) yields dark brown, light green, white, white and dark green complexes. All the complexes are stable at room temperature and melt with decomposition above 250°C.Unfortunately our efforts to obtain single crystals of complexes were not successful. Therefore the ligand, and its complexes were characterized on the basisofelementalanalysis, ^{1}HNMR, IR, magneticsusceptibilitymeasurement,electronicspectradata,colours, melting point, partial elemental analyses and molar conductivities.

The IR spectra of the ligand(viii) show a strong band at 1618 cm $^{-1}$ region for the ν(C=N) stretching (**Howlader M B H et al,. 2007**). The ligand show a strong band at 1645 cm $^{-1}$ and a medium band at 3266 cm $^{-1}$ region for the ν(C=O) and ν(N-H) stretching respectively (**Howlader M B H et al,. 2007**) of the amidic moiety[-C(=O)NH-]. The absence of the ν(N-H) stretching of $–NH_2$ moiety in the ligand indicates that condensation has taken place between amine and aldehyde moieties (**Howlader M B H et al,. 2007**). [The ligand precursor (vi) showed two ν(N-H) stretching band at 3205 and 3139 cm $^{-1}$] (**Howlader M B H et al,. 2007**).

The complexes (ixa-ixe) show a strong band at 1607-1610 cm $^{-1}$ band at region, representing the ν (>C=N-N=C<) stretching moiety. This band is shifted to a lower frequency by ~ 10 cm $^{-1}$, as compared to the correspondingligand indicating that the azomethine nitrogen of the C=N group has participated in coordination (**Howlader M B H et al,. 2007**). The complexes show new bands at 598-651 and 517-566 cm $^{-1}$ region, indicative of the ν(M-N) bands of the [-C(=O)NH-N=C<] moiety as seen in the ligands, supported the formation of chelate complexes by losing the amide proton through the enolic form.

The ^{1}H NMR spectra of the ligand (viii) shows three singlet at δ 2.2,(3H) 5.3,(2H) 8.4(1H) and multiplet at 6.9 - 8.6 (12H) for the -CH_3, -OCH_2-, [-N=CH-] and aromatic [-C_6H_4,-C_6H_4Cl] protons indicating the formation of the Schiff base protons respectively. The ligand shows a broad singlet at δ 11.8 for the amidic proton of the [-C (=O) NH-N<] moiety.

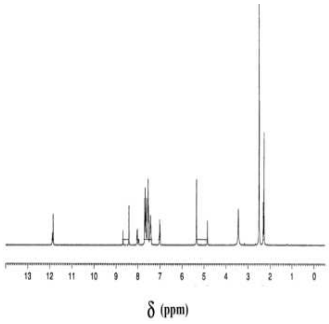

Figure 2: ^{1}H NMR Spectrum of Ligand (viii)

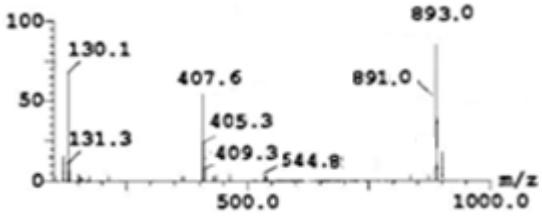

Figure 3: Mass Spectrum of Ligand (viii)

Magnetic Measurements and Electronic Spectra

The magnetic moment of the complexes were measured at room temperature.The magnetic moment of the cobalt (II) lay in the range 3.82-3.90 BM which corresponds to 3 unpaired electrons. The solution spectra of the cobalt (II) complex exhibited absorption in the region 250-260, 310-340, 400-420 nm. The spectra resemble that reported for octahedral complex **(D.P. Singh et al., 2009)**.Thus the various bands can be assigned to: $^4T_1g \rightarrow {}^4T_2g$, $^4T_1g \rightarrow {}^4A_2g$, $^4T_1g \rightarrow {}^4T_1g$.

The magnetic moment of the nickel (II) was lay in the range 2.82-2.96 BM which corresponds to 2 unpaired electrons. The solution spectra of the nickel (II) complex exhibited absorption in the region 320-345, 357-396, 438-480 nm. The lowest energy transition corresponds to the transition

$^1A_1g \rightarrow {}^1A_2g(d_{xy} \rightarrow d_{x^2-y^2})$ which suggests that the energy levels order is $d_{z^2} < d_{xy}$.**(D.J. Mac Donald et al, 1967)**.

The other transitions corresponds to $^1A_{1g} \rightarrow {}^1B_{1g}(d_{z^2} \rightarrow d_{x2-y2})$, $^1A_{1g} \rightarrow {}^1E_g(d_{xy}, d_{yz} \rightarrow d_{x2-y2})$ respectively. These transitions reveal that the nickel complex possess square planar geometry and D_{4th} symmetry **(B.N. Figgis et al, 1966).** Spectra resemble that reported for octahedral complex.

The magnetic moment of the copper(II)lay in the range 1.88-1.93 BM which lie appreciably above the spin-only value of 1.73 BM for Cu(II) ion, indicating the presence of single unpaired electron and suggesting the square planar structure for the complex. The solution spectra of the copper (II) complex exhibited absorption in the region 260-296, 340-393, 460-510 nm. Thus for a square planar complex with $d_{x^2-y^2}$ ground state, three spin allowed transitions are

possible, viz.,$^2B_{1g} \rightarrow {}^2A_{1g}(d_{x2-y2} \rightarrow d_{yz})$, $^2B_{1g} \rightarrow {}^2B_{2g}(d_{x^2-y^2} \rightarrow d_{xy})$ and $^2B_{1g} \rightarrow {}^2E_g(d_{x2-y2} \rightarrow d_{xy}, d_{yz})$. Since the four 'd' orbitals lie close together, the transitions cannot distinguished by their energy and hence, it is very difficult to resolve the bands into separate components **(Tabl ASE et al,2002**).

The zinc(II) and cadmium(II) do not show any d-d transitions and hence electronic spectral measurements have not been done.

Table 1: Analytical, Conductance and Magnetic Moment Data of Compound

Compound	Mol. Formula (Colour)	M.pt. (°C)	Yield (%)	Found (Calc.) %			μ_{eff} (BM)	Λm_2 ($ohm^{-1}cm^2\ mol^{-1}$)
				C	H	N		
L	$C_{23}H_{19}N_2O_3$ white	120-122	80	68.01 (67.91)	4.17 (4.67)	6.90 (6.88)	–	–
M_1	$C_{48}H_{36}N_4O_6Cl_2Co(H_2O)_2$ Dark brown	361	70-74	61.90 (61.94)	4.22 (4.33)	6.11 (6.01)	3.82-3.90	21.54
M_2	$C_{48}H_{36}N_4O_6Cl_2Ni$ Light green	>250	70-74	64.70 (64.46)	4.02 (4.05)	6.32 (6.26)	2.82-2.96	27.20
M_3	$C_{48}H_{36}N_4O_6Cl_2Zn$ white	452	70-74	64.00 (63.98)	4.11 (4.02)	6.01 (6.21)	–	11.10
M_4	$C_{48}H_{36}N_4O_6Cl_2Cd$ white	383	70-74	60.01 (60.81)	4.01 (3.82)	5.98 (5.91)	–	13.20
M_5	$C_{48}H_{36}N_4O_6Cl_2Cu$ Dark green	>250	70-74	64.23 (64.11)	4.11 (4.03)	6.15 (6.23)	1.88-1.93	23.20

Thermal Studies

The TGA curve of the M_1-M_5 complexes were carried out within a temperature range from room temperature up to 800 °C. The thermal decomposition data of complexes were listed in Table 2. The TGA and DTG curves of complexe M_5 presented in Figure 4. The data from thermogravimetric analysis clearly indicated that the decomposition of ligand molecules were lost in between 250-380 °C. Finally, the metal oxides were formed above 400 °C. The decomposition was completed at ≥800 °C.

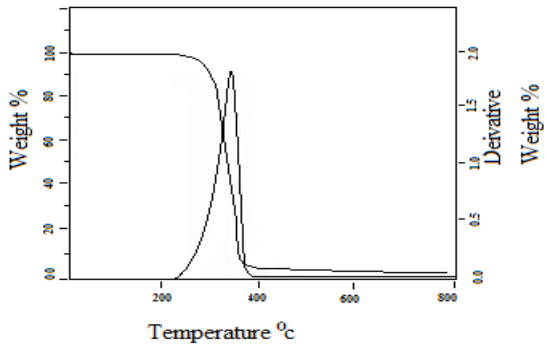

Figure 4: TGA Curve Of Cu(II)Complex (M5)

Table 2: Stepwise Thermal Degradation Data obtained from TGA Curves and their Composition

Complex	Temp. range (°C)	Degradation Products	No. of Moles	% Weight loss		% Residue	
				Calcd	Expt	Calcd	Expt
M_1	125-220	H_2O	2	3.87	3.82	6.34	6.18
	180-460	Ligand	2	89.79	89.9		
M_2	200-500	Ligand	2	93.43	93.1	6.56	6.9
M_3	200-470	Ligand	2	92.74	92.5	7.26	7.5
M_4	180-390	Ligand	2	88.14	88.10	11.86	11.9
M_5	250-380	Ligand	2	92.93	92.9	7.06	7.1

Biology

Choriallanotoic Membrane (Cam) Assay

Figure 5: Suppression of Angiogenesis in Vivo by Synthetic Novel M_1, M_2, M_4 And M_5 in Shell Less CAM Assay. Decreased Vasculature was Observed in Treated Groups Compared to Control

In the present investigation anti angiogenic activity of M_1, M_2, M_4 and M_5 showed reduced proliferation of blood vessels in the shell less CAM assay model of developing embryos. The proliferation of microvessels regressed around the zone of compounds treated with M_1, M_2, M_4 and M_5 supporting the antiangiogenic activity.

DNA Cleavage Studies by Gel Electrophoresis

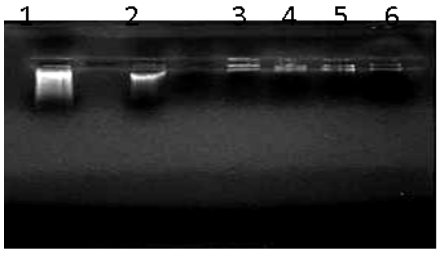

Figure 6: Photograph Showing the Effects of Synthetic Molecules on Calf Thymus DNA. Lane 1: Untreated DNA, Lane 2: Viii, Lane 2: M_1, Lane 3: M_2, Lane 4: M_3, Lane5: M_4.Lane 6: M_5

After binding to DNA, synthetic molecule can induce several changes in DNA conformation. Synthetic molecules, which could induce DNA deformations, such as bending, 'local denaturation' (over winding and under winding), intercalation, micro loop formation and subsequent DNA shortening lead to decrease in molecular weight of DNA. Gel electrophoresis is an extensively used technique for the study of binding of compounds with nucleic acids: in this method segregation of the molecules will be on the basis of their relative rate of movement through a gel under the influence of an electric field. DNA is negatively charged and when it is placed in an electric field, it migrates towards the anode; the extent of migration of DNA is decided by the strength of the electric field, buffer, and density of agarose gel and size of the DNA. Generally it is seen that mobility of DNA is inversely proportional to its size. Gel electrophoresis picture is shown in figure 6.The photographs reveal the bands with different band width and brightness compared to the control.

The difference observed in the band width and intensity is the criterion for the evaluation of binding/cleavage ability of synthetic molecule with calf thymus DNA. Figure 5. shows the bands with different band width and brightness compared to control. There is significant binding/cleavage of DNA in the lane 3, 4, 5 and 6 compared to the control (lane-1) and ligand (lane-2) indicating that these molecules strongly bound to the DNA and degraded it there by decreasing the intensity and the band width.

CONCLUSIONS

The work has approached towards the synthetic and biological approach of Schiff base and its metallic complexes. The Elemental analyses confirms the chemical composition of the synthesized compounds while FT-IR and ^{1}H-NMR spectroscopy confirms the functional groups, particularly -HC=N, C=O and -C(=O)NH-N=C< groups, of the compounds. All spectroscopic analysis confirmed the proposed structures for these compounds. Biological data have shown that the synthesized compounds have a significant biological activity. There is significant binding/cleavage of DNA in the lane 3, 4, 5 and 6 compared to the control and ligand indicating that these molecules strongly bound to the DNA and degraded it there by decreasing the intensity and the band width. The anti angiogenic activity of M_1, M_2, M_4 and M_5 showed reduced proliferation of blood vessels in the shell less CAM assay model of developing embryos. The proliferation of microvessels regressed around the zone of compounds treated with M_1, M_2, M_4 and M_5 supporting the anti angiogenic activity.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with respect to the content of the manuscript.

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